

REMARKS

This amendment does not introduce new subject matter as support is found in the application as filed.

A copy of the change of the paragraphs on pages 50, 59 and 60 is attached hereto.

Applicant's request that the Attorney Docket Number for this matter be changed to 112408.122 on all future communications from the Patent Office.

No fees are believed to be due with the filing of this Sequence Listing. The Commissioner is hereby authorized to debit Deposit Account No. 08-0219 any required fee necessary to maintain the pendency of this application.

Respectfully submitted,

Date: October 16, 2002

Hollie L. Baker

Hollie L. Baker
Registration No. 31,321
Attorney for Applicant

HALE AND DORR LLP
60 State Street
Boston, MA 02109
Tel: (617) 526-6110
Fax: (617) 526-5000

Example 1

Generation of Synthetic Protein Libraries and Modification by Tissue

The starting point was a cDNA library preparation from "marathon-ready" human colon cDNAs obtained from Clontech UK Ltd (Basingstoke, UK). The library was amplified by PCR using primers AP1 (SEQ ID NOS.: 62 and 63) [5'ccatcctaatacgactcaactataggc] and AP3 [5'ttctagaattcagccgc(t)₃₀nn] using the Advantage cDNA PCR kit and conditions recommended by the supplier (Clontech). The resultant PCR product was cloned using a T/A cloning strategy into SmaI linearised pUC19 prepared according to Marchuck et al (Marchuck D. et al 1991, *Nucl.Acids Res.* **19**: 1154). Alternatively, a human colon cDNA library constructed from human colon mRNA isolated directly from tissue using standard methods described in *Molecular Cloning, A Laboratory Manual* eds. Sambrook J, Fritsch EF, Maniatis T. Cold Spring Harbor Laboratory Press 1989, New York, USA. The mRNA converted to cDNAs using the RNase H method (described in *Molecular Cloning* ibid) and recloned into the SmaI site of pGEMT7/SP6 plasmid (Promega, Southampton, UK) providing a promoter for T7 RNA polymerase. Long synthetic oligonucleotides and PCR were applied to provide an upstream bacterial ribosome binding site, a downstream spacer derived from the M13 phage gene III and a 3' transcriptional terminator region from the *E. coli* lpp terminator as described in Hanes and Pluckthun, *Proc. Natl. Acad. Sci.* **94** (1997), p4937.

The library was diluted 1 in 5 in ice-cold dilution buffer (50mM Tris Acetate pH 7.5, 150 mM Sodium Chloride, 50 mM Magnesium Acetate, 0.1% Tween 20 with 200u/ml of RNasin (Promega UK Ltd, Southampton UK). 50 μ l of the diluted library was added to each well of the IVTT protein array and incubated at 4°C for 1 hour with gentle shaking. Following incubation, wells of the array were washed 5 times using 100 μ l ice-cold dilution buffer. The mRNA from any bound mRNA/ribosome/protein complexes was eluted by addition of 20 μ l of 50 mM Tris acetate pH 7.5, 150 mM Sodium Chloride, 20 mM EDTA and incubating on ice for 10 minutes with gentle shaking. Eluted mRNA was collected into a second 96 well plate and concentrated by precipitation with ethanol at -20°C overnight (Molecular Cloning, A Laboratory Manual ibid) in the presence 20 μ g of glycogen carrier, (Boehringer, Lewes, UK). The resulting mRNA was used as a template for reverse transcription with M-MLV reverse transcriptase (RT). Briefly, mRNA was heat denatured at 70°C for 10 minutes in the presence of 1 nM of the primer RD3 [5'(T)₁₈nn]. The mixture was chilled on ice before addition of 200u M-MLV-RT and its reaction buffer (Life Technologies, Paisley, UK). The mixture was incubated for 60 minutes at 37°C then shifted to 70°C for 15 minutes to inactivate the RT. The subsequent DNA:RNA hybrid was used as a template for PCR, using primers T75L (SEQ ID NO.: 64) [5'cggttccctctagaaata] and RD3 and cycled x 40 at 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds. Thermostable DNA polymerase (ExpandTM High Fidelity), reaction buffer and nucleotide triphosphates were from Boehringer (Boehringer, Lewes, UK). PCR products were analysed by agarose gel electrophoresis (Molecular Cloning, A Laboratory Manual, ibid). The presence of a PCR product identified putative positive binding partners in the IVTT protein array. The protein array was de-coded and phagemid DNA identified for sequence analysis. PCR products from the polysome display library were sequenced directly using primer T75L. Sequencing was conducted using dye-terminator chemistry according to protocols provided by the supplier (Amersham International, Amersham UK). Reactions analysed using an automated sequencing system (Applied Biosystems, Warrington, UK).